

Sleep

A Functional Enigma

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Abstract

Although continued total sleep deprivation is fatal, the function of sleep remains a mystery. Shorter durations of sleep deprivation are followed by rebound increases in non-rapid eye movement (non-REM) sleep, suggesting a homeostatic control. Measurements of the power spectrum of the electroencephalograph (EEG) suggest that a more accurate marker of the homeostasis may be δ frequency power, because it most closely reflects the duration of the preceding sleep deprivation. Several lines of evidence suggest a link with complex metabolic processes. These include a local homeostatic factor, adenosine, that inhibits neuronal activity in response to increases in the ratio of energy demand to metabolite availability. Other evidence derives from the relationship of circadian genes, NPAS2 and Clock, to metabolism. Additionally, at a systems level, hypocretin/Orexin may coordinate motor activity with feeding. A loss of hypocretin neurons or a mutation of the genes controlling this peptide system can result in the sleep disorder narcolepsy.

Finally, evidence for a role of non-REM sleep in developmental central nervous system (CNS) plasticity, as well as learning and memory, is discussed.

The function and control of sleep in animals remains one of the most elusive biological enigmas. Because the behavioral manifestations of sleep that characterize this state, including motor inactivity,

decreased or delayed responsiveness to sensory stimuli, and rapid return to normal waking levels of these behavioral parameters with arousal (Shaw et al., 2000; Shaw and Franken, 2003), are depen-

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dent on altered central nervous system (CNS) activity in vertebrates and probably invertebrates as well, sleep is an enigma of neurobiology. In higher vertebrates, this characteristic behavioral change is correlated with a change in the activity pattern of the majority of neurons in the CNS, as suggested by an altered electroencephalograph (EEG) waveform. The waking EEG shows predominantly low-amplitude but high-frequency components compared to a sleeping EEG (the non-rapid eye movement [non-REM]), with predominantly high-amplitude components, especially in the 0.5–5 Hz range. The other neuronal characteristic of non-REM sleep is a slowing of the CNS action potential firing rates compared to waking levels (Evarts, 1967). In addition, mammals also have CNS state transitions from non-REM to REM sleep. REM sleep is similar to waking regarding the firing rates of most neuronal cell types, as reflected by the EEG. REM is dramatically different, because in this stage, "waking-like" EEG activity is associated with a profound somatomotor inhibition and a reduced responsiveness to sensory stimuli. Neither the reason for these changes (i.e., the function subserved by them) nor the neurobiological mechanism(s) responsible are understood, but some potentially important clues constraining the answers to these questions have emerged from recent studies that follow.

Sleep Deprivation

The traditional approach employed in the investigation of the function of sleep has been to examine the outcome of sleep deprivation, but even this seemingly straightforward approach is not without controversy. Much of it stems from the phenomenological definition of non-REM sleep as a group of temporally associated phenomena that usually includes an EEG with predominant slow wave activity and reduced sensory and motor activity, as indicated by a less active EMG and reduced responsiveness to sensory stimuli. Although this group of phenomena is distinct between normal or average behavioral states of waking and non-REM sleep, when perturbations from the norm occur, then the distinctions break down. This happens with severe sleep deprivation, brain lesions, or pharmacological manipulations (e.g., the intrusion of δ wave activity into the waking EEG). Furthermore, these

distinctions cannot be directly applied to non-mammalian species, because the EEG and electromyogram (EMG) are qualitatively different for waking and nonwaking behavioral states.

One important indicator for sleep function is suggested by the rebound increase in sleep that follows sleep deprivation. Although the relationship between sleep and the preceding sleep deprivation is variable, the relationship of the slow wave activity during sleep after sleep deprivation (as measured using a fast Fourier analysis of the EEG power in the δ frequency range of 0.5–4 Hz) is correlated with the duration of sleep deprivation (Borbely, 1982; Franken et al., 1991; Tobler and Borbely, 1986). This relationship is even more robust if one focuses entirely on the delta power deprivation and rebound (Franken et al., 1991). The δ power increases results from a combination of increased activity and synchronization of CNS electrical activity within the δ frequency range. At a cellular level, this probably reflects a coordinated burst-pause firing in the thalamus and cortices. The burst-pause oscillation is intrinsic to many thalamocortical neurons and is mediated by an interaction of the hyperpolarization-activated current (I_h), the transient calcium current (I_t), and a resting membrane potential in the absence of I_h of approx -80 mV (McCormick and Bal, 1997; McCormick and Pape, 1990). Whether this is only an indicator of sleep homeostasis or rather a mechanism that serves an integral role remains to be answered. Predictably, the burst-pause activity will be associated with a characteristic oscillation at the δ frequency of intracellular calcium concentration. It is conceivable that this particular intracellular calcium dynamic signals or at least modulates gene expression and/or second messenger activity.

What happens with prolonged total sleep deprivation? Rats develop a progressively more debilitated appearance, skin lesions, and increased energy expenditure until 11–32 d of deprivation, at which point they die (Everson et al., 1989). Although the energy expenditure is increased, the food intake is also increased and overall energy use was not significantly changed and neither are glucocorticoid nor thyroid hormonal levels (Bergmann et al., 1989). Gene array analysis shows an altered expression of numerous genes, mainly those involved in intermediate carbohydrate metabolism, protein metabolism, and mitochondrial organogenesis and

function (Cirelli, 2002; Cirelli and Tononi, 2000; Terao et al., 2003). Surprisingly, with prolonged sleep deprivation, most of the upregulated gene expression returns to baseline or is slightly depressed with the exception of Arylsulfotransferase in the mammal or its homolog in *Drosophila* (Cirelli, 2002; Cirelli and Tononi, 2000; Tranque et al., 1996). Furthermore, there is no evidence from the TUNEL technique of cellular degeneration after 14 d of sleep deprivation (Cirelli et al., 1999), although increased amino cupric silver staining occurs, which is indicative of a loss of membrane integrity (Eiland et al., 2002).

A major advance in our understanding of sleep function used a *Drosophila melanogaster* model of sleep (Hendricks et al., 2000; Shaw et al., 2000). Quiet rest (QR) cannot be distinguished from non-REM sleep with traditional means, because the EEG measure of increased slow wave (in the δ frequency range) activity cannot be measured. However, whatever CNS arousal is necessary to sustain the coordinated somatomotor and sensory activity needed to respond to the "gentle tapping or shaking" used to prevent rest is also sufficient to elicit a homeostatic rest rebound, similar to the sleep or δ activity rebound observed in mammals in response to sleep deprivation.

Sleep and Circadian Factors

Shaw and colleagues (2002) have shown that *Drosophila* with a single mutation of the circadian gene, *cyc* (a homolog of the BMAL circadian gene in mammals) or of the heat shock protein (Hsp83) had a strikingly altered response to QR deprivation. The rebound response was greatly exaggerated, and, if the deprivation was prolonged, then the effects were lethal as compared to no lethality in wild-type flies. Finally, preexposure to heat was protective against the lethal effects of sleep deprivation in *cyc* mutants. Thus, a rest/activity cycle homeostasis was demonstrated for nonvertebrates, controlled by specific genes with vital function, at least regarding the survival of sleep deprivation. Perhaps of even greater import was that these findings suggest a functional interaction between circadian and homeostatic controls of rest/activity.

The interaction between circadian and homeostatic mechanisms of rest/activity control may also

be affected by the NPAS2 transcription factor. In the forebrain of mammals, NPAS2 acts as a functional analogue of the circadian gene, *Clock*, and, either *Clock* or NPAS2 may bind to form a heterodimer with BMAL1 to activate transcription of downstream circadian gene products, including *Per1*, *Per2*, and *Cry1* (Reick et al., 2001). However, the activity of either *Clock*-BMAL1 or NPAS2-BMAL1 heterodimer is under tight control of the redox state of the cell (Rutter et al., 2001). Its activity is greatly facilitated by the reduced state of NADP(H) and NAD(H) and inhibited by the oxidized state. Because the redox state is affected by neuronal activity, prolonged neuronal activity might be expected to feedback onto circadian controlled activities. In fact, sleep deprivation by gentle handling in the absence of increased exercise reset circadian phase in the Syrian Hamster, possibly in relationship to the prolonged increased neuronal activity of waking and an associated altered redox state (Antle and Mistlberger, 2000). Thus, prolonged sleep deprivation might contribute to a disruption of the normal circadian progression of gene expression under the control of the NPAS2/BMAL1 dimer that, in the absence of prolonged waking, occurs in accordance with the expected circadian rest/activity cycles.

Adenosine

Adenosine is another potential factor that might mechanistically link metabolism to neuronal sleep activity. The extracellular adenosine concentration increases whenever the ratio of metabolite demand to metabolite availability increases. The increased concentration can inhibit neuronal activity through adenosine A1 receptors, reducing metabolic demand, and thus providing a negative feedback homeostasis between metabolic demand and neuronal activity (Greene and Haas, 1991). In addition, A1 receptor activity may facilitate δ frequency oscillations (Fig. 1) by (1) a combination of increased G-protein dependent inwardly rectifying potassium (GIRK) channel conductance and decreased activation of the hyperpolarization-activated current, *Ih* (Pape, 1992), (2) presynaptic inhibition resulting in a functional deafferentation of the thalamocortical circuits that increases the influence of the GIRK and *Ih* currents to facilitate δ frequency oscillations, and (3) a reduction of cholinergic tone, which inhibits

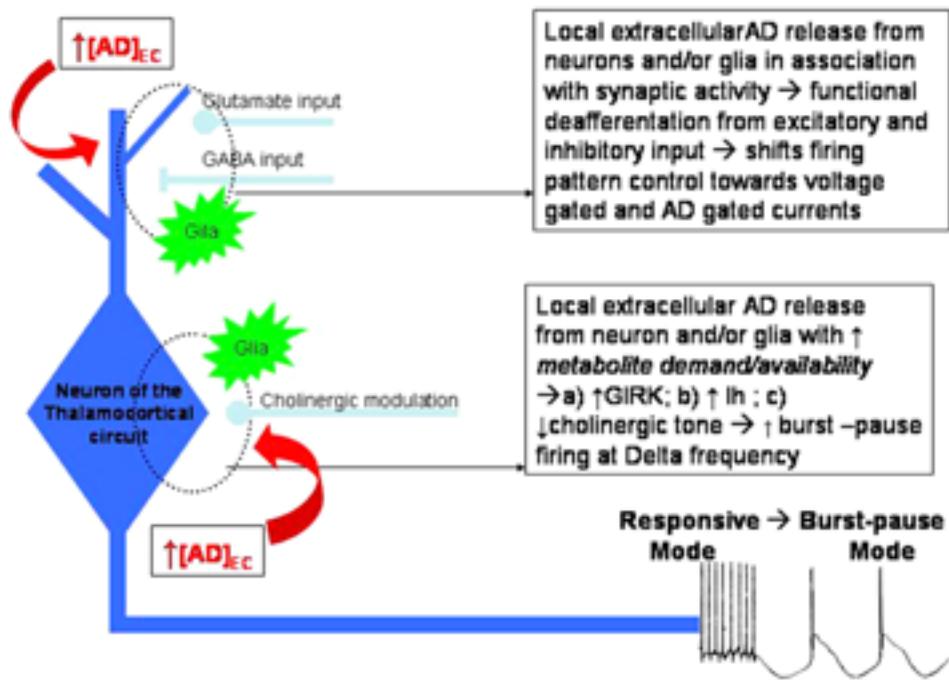


Fig. 1. Adenosine facilitates δ frequency activity in thalamocortical circuits. Color image available for viewing at www.humanapress.com.

burst-pause firing patterns (McCormick, 1993) by acting at both the cholinergic nuclei (Porkka-Heiskanen et al., 1997; Portas et al., 1997; Rainnie et al., 1994) or locally at the terminal fields of the cholinergic thalamocortical targets (Materi et al., 2000). As noted, this δ frequency activity correlates strongly with sleep homeostatic response to sleep deprivation, and it is conceivable that this kind of neuronal activity is especially facilitated in regions of high adenosine concentration resulting from increased neuronal activity. With sleep deprivation and the associated increase in duration of the neuronal of waking, the extracellular concentration of adenosine increases in the thalamus (Porkka-Heiskanen et al., 1997) and in the cortex (Porkka-Heiskanen et al., 2000). The CNS state of non-REM sleep might be considered permissive to δ wave activity that would be facilitated by the increased adenosine. Others have speculated that adenosine's role in sleep relates to both δ wave activity (Benington et al., 1995) and its induction of glycogenolysis because glycogen stores may be depleted during prolonged waking (Benington and Heller, 1995; Kong et al.,

2002). This is consistent with adenosine's role in energy metabolism to induce glycogenolysis under conditions of increased neuronal activity (Magistretti et al., 1986).

Hypocretin

Another link between sleep and metabolism is suggested by the fasting-induced arousal observed in mammals. Yamanaka et al. (2003) have reported that hypothalamic hypocretin (Hcrt) (also called Orexin) neurons regulate arousal according to energy balance in studies of Hcrt knockout mice. They find that these mice fail to respond to fasting with increased activity and wakefulness. In prior studies Wu et al. (2002) reported that Hcrt levels were not altered by 48-h food deprivation nor by food consumption in normal dogs. However, when these same dogs were allowed to exercise in a large yard, Hcrt levels increased by an average of 70%. These observations can be reconciled by the hypothesis that Hcrt is more closely linked to aspects of

motor activity (and the associated neuronal activity) than to peripheral energy balance *per se* and that food deprivation in mice under the conditions of Yamanaka et al.'s studies increased motor activity, whereas it did not under the conditions of Wu et al.'s study.

Many of the symptoms of narcolepsy can be mimicked by genetic deletion of the hypocretin or Orexin peptide in mice (Chemelli et al., 1999) or the mutation of the hypocretin or Orexin receptor2 gene in the dog (Lin et al., 1999). In 2001, it was discovered that most human narcolepsy was caused by a loss of hypothalamic cells containing Hcrt (also called Orexin) (Peyron et al., 2000; Thannickal et al., 2000). It was also found that administration of the peptide to genetically narcoleptic dogs reversed symptoms of the disorder (John et al., 2000), suggesting that similar treatment of human narcoleptics could be a uniquely effective treatment for narcolepsy.

In further work in normal animals, Hcrt was released maximally in waking and REM sleep and minimally in non-REM sleep (Kiyashchenko et al., 2002). In waking, its release was linked to certain kinds of motor activity, leading to the hypothesis that release of Hcrt facilitates motor activity during emotionally charged activities of the sort that usually trigger cataplexy (Eiland et al., 2002; Gulyani et al., 2002; Siegel, 2003a; Wu et al., 2002). Even normal individuals experience weakness at these times, which is seen in the "doubling over" that often accompanies laughter or the weakness that can result from sudden-onset, strong emotions. In the absence of the Hcrt-mediated motor facilitation, muscle tone is lost. Many Hcrt neurons also have ascending projections to link cortical arousal to motor and emotional activity. In the absence of Hcrt-mediated facilitation of forebrain arousal centers, waking periods are truncated, resulting in the sleepiness of narcolepsy (Siegel, 2003b; Siegel, 1999; Siegel, 2003a).

Hcrt acts largely by modulating the release of amino acid neurotransmitters (van den Pol et al., 1998). Systemic injection of Hcrt causes a release of glutamate in certain regions innervated by Hcrt, producing a potent postsynaptic excitation (John et al., 2003; Peever et al., 2003). In other regions, it facilitates γ -aminobutyric acid (GABA) release, producing postsynaptic inhibition (Kiyashchenko et al., 2002; Liu et al., 2002). The loss of these competing inhibitory and facilitatory influences leaves brain motor regulatory and arousal systems less stable

than the tightly regulated balance that can be maintained in the presence of Hcrt (see Fig. 2). According to this hypothesis, this loss of stability is the underlying cause of narcolepsy, resulting in loss of muscle tone in waking, inappropriate increases of muscle tone during sleep, resulting in a striking *increased* incidence of REM sleep behavior disorder in narcoleptics (Schenck and Mahowald, 1992). Likewise, although a principal symptom of narcolepsy is intrusions of sleep into the waking period, people with narcolepsy sleep poorly at night with frequent awakenings (Guilleminault and Anognos, 2000; Siegel, 1999; Siegel, 2000). In other words, people with narcolepsy are not simply weaker and sleepier than normals. Rather, their muscle tone and sleep-waking state regulation is less stable than that in normal persons. The authors hypothesize that this results from the loss of the simultaneous inhibitory and excitatory actions of Hcrt.

Sleep, Plasticity, and Learning and Memory

Sleep deprivation alters cortical plasticity during development. Occlusion of one eye in the cat for as little as 6 h results in significant plastic changes in the visual thalamocortical circuitry during a critical developmental period for ocular dominance. Sleep deprivation reduces the remodeling induced by the monocular occlusion (Frank et al., 2001). As the authors note, this finding does not imply a particular mechanism for the sleep deprivation effects, nor was it possible to correlate the amount of sleep with the degree of plasticity (altered response). More importantly, it cannot be determined whether the sleep-induced reduction reflects a direct effect on mechanisms responsible for the remodeling or some indirect effect permissive to the remodeling process. For example, this process is depend on protein synthesis (Taha and Stryker, 2002), so that any changes in protein metabolism might have an effect. Similar concerns may apply to the interpretation of the effects of sleep deprivation on consolidation or reconsolidation of a procedural learning and memory task.

In humans, memory can be subdivided into declarative and procedural forms. Declarative memory is fact-based information of the sort that we learn in school or information acquired during

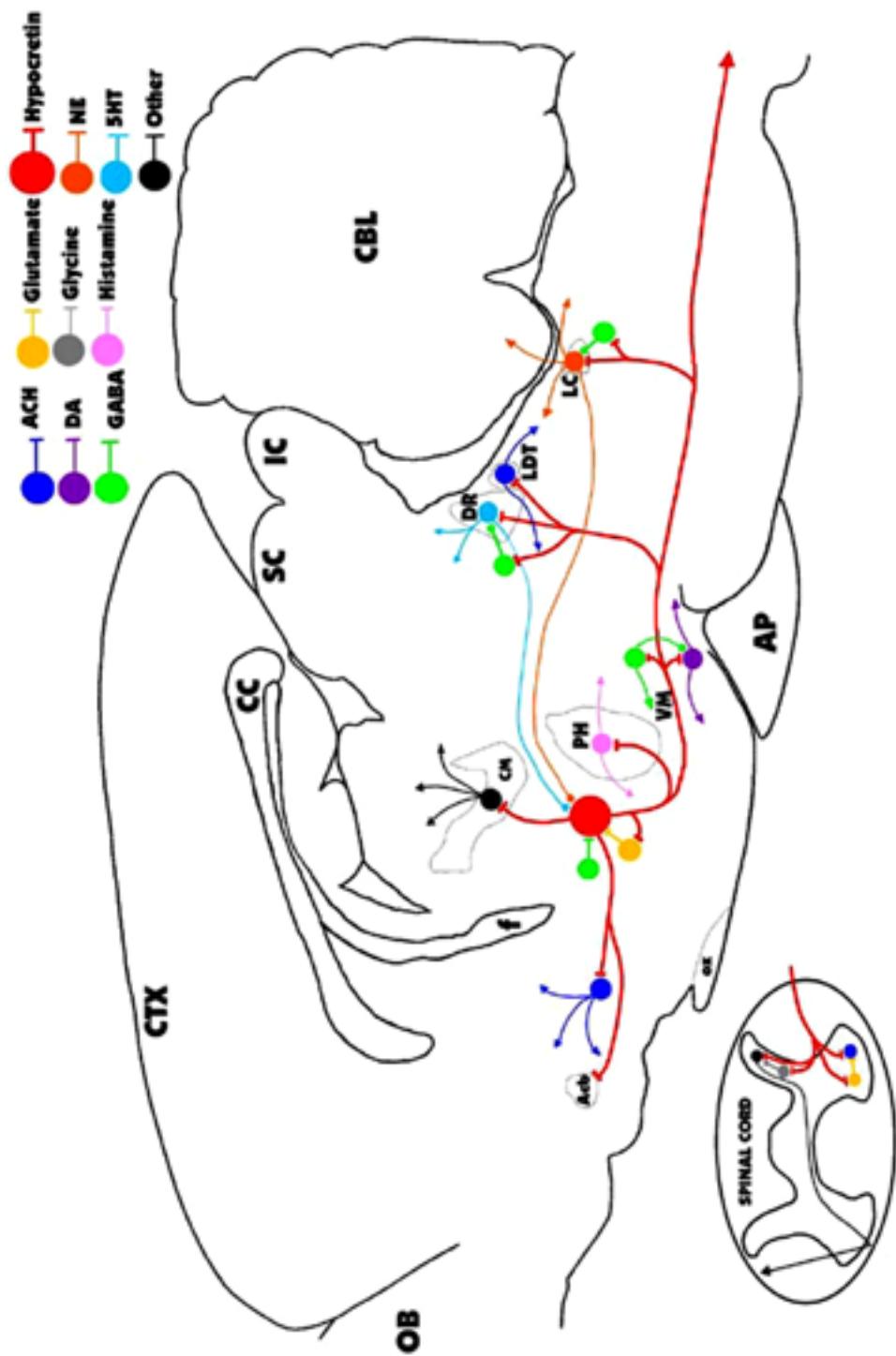


Fig. 2. Major identified synaptic interactions of hypocretin (Hcrt) neurons. Lines terminated by perpendicular lines denote excitation; circular terminations indicate inhibition. ACH, acetylcholine; DA, dopamine; NE, norepinephrine; 5HT, serotonin; OB, olfactory bulb; Acb, nucleus accumbens; f, fornix; OX, optic chiasm; CM, centromedian nucleus of the thalamus; PH, posterior hypothalamus; VM, ventral midbrain; AP, anterior pituitary; SC, superior colliculus; IC, inferior colliculus; DR, dorsal raphe; LDT, laterodorsal tegmental and pedunculopontine; LC, locus coeruleus; CBL, cerebellum. Color image available for viewing at www.humanapress.com.

daily activities. Declarative memory uses the hippocampus and adjacent temporal lobe structures, and, therefore, these structures have been the focus of physiologically based sleep-learning experimenters, including those observing neuronal unit activity during sleep (Louie and Wilson, 2001). Procedural memory is a nonconscious learning, such as improvement of perceptual or motor skills as might occur in learning to ride a bicycle or to play a musical instrument.

Initial sleep learning studies revolved around that concept that dream activity represented a rehearsal of declarative tasks and that REM sleep was critical to declarative memory. However, accumulated evidence demonstrating that neither REM sleep nor total sleep loss interfered with learning when nonstressful sleep deprivation was employed has led critics to question the evidence for a role for sleep in declarative learning (Siegel, 2001; Vertes and Eastman, 2000). Recently, even a leading advocate of a role of sleep in learning has concluded that there is no substantial role for either REM or non-REM sleep in declarative learning (Smith, 2001).

Therefore, most current sleep-learning work has focused on the idea that sleep may have a role in procedural learning. However, evidence for an important role of sleep in procedural learning has also been inconsistent. Studies supporting a role for sleep in the consolidation of human procedural learning have made contradictory claims about similar learning tasks, with some concluding that REM but not non-REM sleep is important, others stating just the reverse, others claiming that both sleep states are essential (Siegel, 2001; Stickgold et al., 2000b), and still others making ad hoc claims, such as that only stage 2 non-REM sleep in the last quarter of the night is important (Walker et al., 2002), which is disputed by procedurally similar studies claiming that REM sleep rather than stage 2 was important (Fischer et al., 2002). Therefore, despite great interest in the area, the evidence for a sleep-learning connection remains weak. Nonetheless, if a subject is allowed to sleep in the hours after training (e.g., 8 h) on certain simple sensory motor tasks the performance improves, despite the absence of any further training (Stickgold et al., 2000a; Walker et al., 2003). Furthermore, performance on this task after reexposure to it can also be affected by subsequent sleep or the lack of it. The mechanisms that might be responsible remain to be examined. This may be

a form of consolidation and / or reconsolidation, but the mechanisms are distinct from the consolidation or reconsolidation of either episodic (or in humans, declarative) or emotional, learning and memory, because different neuroanatomical systems are involved with procedural learning and memory. A phenomenon that could involve procedural memory systems is the increased cortical responsiveness observed in non-REM sleep after slow (approx 1 Hz or less) oscillations that lasts for minutes (Steriade and Timofeev, 2003), although the involvement of plasticity lasting for several minutes should not be confused with consolidation, itself. Furthermore, the existence and characterization of consolidation and reconsolidation processes for these kinds of procedural memory is not well established.

Neurons located in the hippocampus fire action potentials in correlation with an animal's location (hence termed place cells). Remarkably, the correlation of the firing of multiple place cells changes with repeated exposure to and exploration of a particular spatial environment such as a maze, and these changed correlations of firing may be observed during sleep (Skaggs and McNaughton, 1996; Wilson and McNaughton, 1994) in conjunction with sharp wave activity in the hippocampus (Lee and Wilson, 2002; Nadasdy et al., 1999; Ylinen et al., 1995). It is noted that the relationship between place cells and spatial learning is not well understood (McHugh et al., 1996) much less the relationship of this kind of correlated firing of multiple place cells. Nevertheless, these kinds of activity are consistent with a permissive or facilitative role of sleep in spatial memory, but the sharp wave activity is not restricted to sleep (Buzsaki et al., 1992). This hippocampal activity does raise the possibility of a role in consolidation of episodic memory traces (Buzsaki, 1998; Csicsvari et al., 2002; Nadasdy et al., 1999). What is clear is that adequate sleep is vital to the performance of a wide range of tasks, whether these tasks are well consolidated or recently learned.

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